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Further study on fructooligosaccharides of *Rhodobryum ontariense*

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Abstract – The identification of nystose and 1^F-ß-fructofuranosylnystose in the oligosaccharide-rich fraction of the moss *Rhodobryum ontariense* (Kindb.) Kindb. (Bryaceae) by thin layer chromatography and high pressure chromatography with anion exchange-pulsed amperometric detector is discussed. According to our knowledge, it is the first record of these saccharides in the genus *Rhodobryum* as well as in the group of mosses as a whole. The findings are in good agreement with the recent isolation of 1-kestose from the same plant material and confirm that short-chain fructooligosaccharides, well known as prebiotics, are present in the examined bryophyte.

***Rhodobryum ontariense* / Serbia / Phytochemistry / Nystose / 1^F-ß-Fructofuranosylnystose**

INTRODUCTION

One is surprised to note how little information is available on the sugar constituents of bryophytes in contrast to data on higher plants. Chopra & Kumra (1988) state that carbohydrates are widely presented in bryophytes as mono and polysaccharides. The oligomers and polymers of fructose, the fructans, are reported from liverworts among the rest (Hendry & Wallace, 1993). Leafy liverworts (Jungermamanniales) contain a diverse range of soluble carbohydrates, including sucrose, fructan and polyols such as mannitol, sorbitol and volemitol (Suleiman *et al.*, 1979; 1980; Suleiman & Lewis, 1980). Glucose, fructose, trehalose

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and starch are present in trace amounts in the liverwort *Plagiochila aspleniodes* (L.) Dum. (Suleiman *et al.*, 1979). Moreover, monosaccharides, derived by mild acid hydrolysis of structural carbohydrate polymers consist of galactose and arabinose (major components), glucose, xylose and mannose (present in intermediate quantities) and fucose and rhamnose (minor components). Not all these compounds are found in each species and their presence can be used as a taxonomic character (Suleiman *et al.*, 1980). A homologous series of fructans including the trisaccharide 1-kestose is found in the liverwort *Porella platyphulla* (L.) Lindb (Marschall *et al.*, 1998). The occurence of raffinose, or an allied sugar, in certain bryophytes, in *Psilotum*, is worthy of note as well (Allsopp, 1951). The same author emphasises that free pentoses can be present in very small amounts.

However, the information on soluble sugars of mosses is even more sparce and can be characterised as semiquantitative (Margaris & Kalaitzakis, 1974). The presence of fructosides is indicated by paper chromatography (Black *et al.*, 1955; Chollet & Dufour, 1955; Quillet *et al.*, 1956; Theander, 1954). The soluble carbohydrates of *Sphagnum* are found to consist of glucose, fructose, sucrose, and a series of fructosides. According to Chollet & Dufour (1955), these fructosides account for 90% of the soluble carbohydrates in *Sphagnum palustre*. A survey of 14 species of *Sphagnum* has shown that two tri- and three tetra-saccharides, consisting of fructolysated sucrose, are their regular constituents (Maass & Craigie, 1964). On the other hand, studying the soluble sugars of the mosses *Tortula princeps*, *Rhynchostegium* sp., *Platyhypnidium ripariodes*, *Homalothecium* sp. and *Campothecium* sp., Margaris & Kalaitzakis (1974) have found mannose, melibiose, maltose, and deoxyribose for the first time in these plants.

The genus *Rhodobryum* is rather small, but natural. It comprises four species in the Northern Hemisphere including *Rhodobryum ontariense* (Kindb.) Kindb. In Serbia this moss species grows in forest steppic fragments of Deliblatska sands, where winters are cool with scarce precipitation, while summers are dry and hot. Traditional Chinese medicine suggests that mosses of *Rhodobryum* species can cure cardiovascular diseases as crude drugs (Harris, 2008). Till date, both chemical composition and biological activity of this moss species have been investigated (Pejin *et al.*, 2011a; 2011b; 2012a; 2012b; 2012c). As for saccharides, 1-kestose, a short-chain fructooligosaccharide (FOS) with two fructose units, has been recently isolated from *R. ontariense* (Pejin *et al.*, 2012d); it is the first report for FOS in this moss genus. In order to continue the study on these sugars new chemical screening of this moss has been performed.

MATERIALS AND METHODS

General

^{1}H- and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, on a Bruker Avance-400 spectrometer, using an inverse probe fitted with a gradient along the Z-axis, using the solvent signal as an internal standard. Si gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Reverse phase chromatography was performed using Lobar C-18 column (Merck).

Plant material

The moss *Rhodobryum ontariense* (Kindb.) Kindb. (Bryaceae) was collected in Deliblatska sands NE Serbia in May 2009. A voucher specimen (BEOU-4708) has been deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia.

Isolation of fructooligosaccharide-rich fraction

Air-dried gametophyte tips (60 g) were ground and extracted two times for three hours and one more time overnight with chloroform, choloroform-ethanol 1:1, ethanol and ethanol-water 1:1 respectively at room temperature. The crude ethanol-water extract (14.94 g) obtained after evaporation of the solvent *in vacuo* was fractioned by Lobar RP-18 chromatography, eluting with increased gradient of methanol in water. Sugar-rich fraction no. 6-8 (4.99 g) was further analysed by thin layer chromatography (TLC) and high pressure chromatography with anion exchange-pulsed amperometric detector (HPAE-PAD, Dionex).

TLC

The fraction was analysed by TLC, using buthanol-acetic acid-H_2O (5:4:1) and acetonitrile-H_2O (8:2) as solvent respectively, in comparison with 1-kestose, nystose and 1^F-ß-fructofuranosylnystose as standards. The sugar spots were visualized with α-naphtol.

Dionex

Afterwards, direct comparison of the analysed fraction with authentic FOS samples was done by Dionex equipped with a Carbopac PA1 column eluted with 15 mM NaOH (1 ml min^{-1}).

RESULTS

The investigated fraction, obtained after reverse phase chromatography, was classified as an oligosaccharide-rich sample by means of its spectroscopic data and typical chromatographic profile. TLC allowed us to identify nystose (1,1-kestotetraose, GF_3; Fig. 1) and 1^F-ß- fructofuranosylnystose (1,1,1-kestopentaose, GF_4; Fig. 2), but not 1-kestose (1-kestotriose, GF_2; Fig. 3). The presence of these short-chain FOS was undoubtedly confirmed by direct comparison with their standards using Dionex.

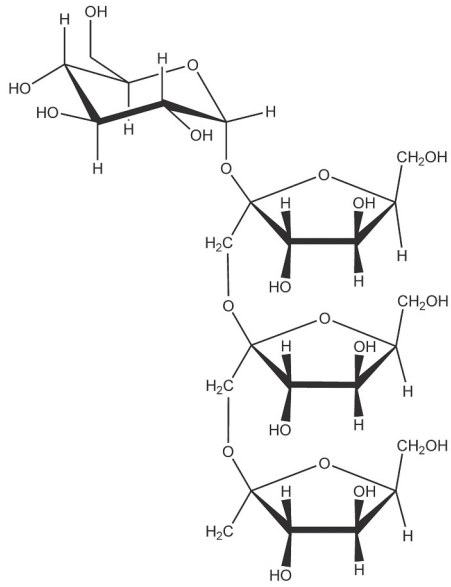

Fig. 1. Nystose.

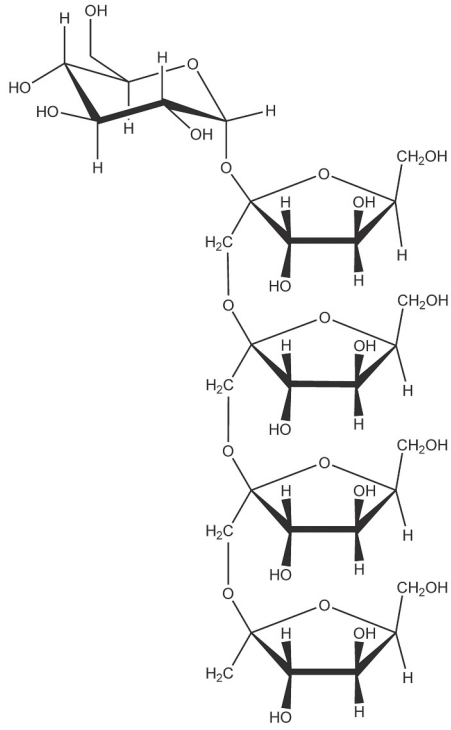

Fig. 2. 1^{F}-ß-Frucofuranosylnystose.

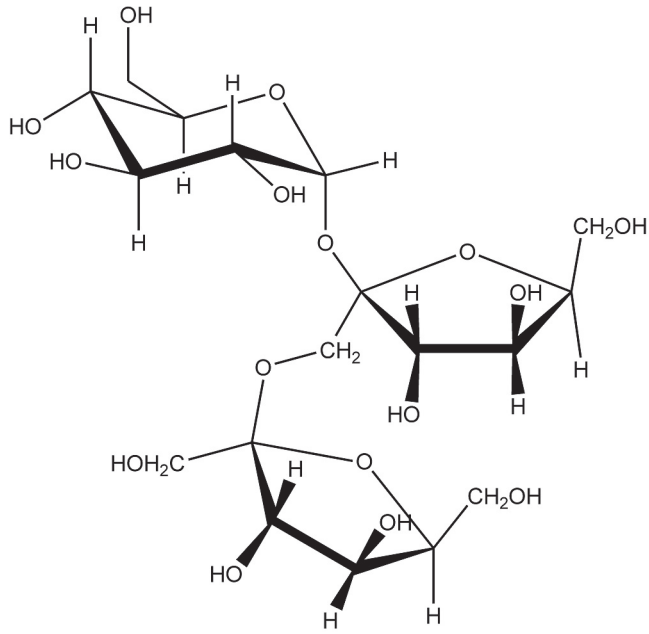

Fig. 3. 1-Kestose.

DISCUSSION

The findings in the scope of this study are in a good agreement with our previous ones and confirm that short-chain FOS are present in the moss *R. ontariense*. Since the isolation and identification of fructose from *Rhodobryum roseum* has been recently reported (Wang *et al.*, 2008), the presence of FOS in other *Rhodobryum* species cannot be excluded. Fructans are considered to be prebiotics regardless of their size and type of linkages and can be used as nutraceuticals for the reduction of serum cholesterol, an increase in calcium and magnesium absorption and the production of B-vitamins (Ritsema & Smeekens, 2003; Van der Meulen *et al.*, 2004; Waterhouse & Chatterton, 1993). Indeed, FOS are recognized as cardioprotective food supplements and is reasonable to be assumed as one of bioactive components of *R. ontariense* medicinal tea traditionally used for hypertension and broad range of heart disorders. However, more studies should be undertaken for the better understanding of *R. ontariense* sugar chemistry and its uses in medicine. Therefore, both chemical and *in vivo* studies on its polar fraction are in progress in our labs.

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